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CONTAINS NO CBI

September 7, 2004

Dear Sir or Madam:

I would like to bring to your attention a recent study completed by my research group on the environmental fate of the antimicrobial compound triclocarban (CAS 101-20-2; the compound is also known as TCC or 3,4,4'-trichlorocarbanilide). We studied the occurrence of triclocarban in urban streams of the Greater Baltimore area and found concentrations of the antimcirobial compound at levels of up to 6.75 ppb. Triclocarban was found in all six urban streams monitored. Results of our study indicate that triclocarban persists in the environment and is a potential contaminant of drinking water resources. More details of our study can be found in the attached paper, published online in *Environmental Science & Technology*, and titled, "Analysis of Triclocarban in Aquatic Samples Using Liquid Chromatography Electrospray Ionization Mass Spectrometry." It will appear in print in the October issue of *ES&T*, Volume 38 (19).

Triclocarban is currently being reviewed under the HPV Chemical Challenge Program of the U.S. EPA. The information contained in our paper should prove helpful for addressing some of the questions that were left unanswered by the robust summary report provided by the chemical industry. In summary, the concentrations found in the Greater Baltimore area are 20-fold higher than those reported previously by the TCC consortium.

Please feel free to contact me by phone (410-955-2609) if you have questions or would like to obtain additional information. My research group is currently conducting a nationwide study on the fate of triclocarban and other pharmaceuticals and personal care products in the environment. We will make every effort to provide the collected information to the U.S. EPA in a timely fashion.

Respectfully,

Rolf Halden, PhD, PE, Assistant Professor of Environmental Health Sciences
Johns Hopkins University Center for Water and Health

Protecting Health, Saving Lives—*Millions at a Time*

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Analysis of Triclocarban in Aquatic Samples by Liquid Chromatography Electrospray Ionization Mass Spectrometry

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Triclocarban, *N*-(4-chlorophenyl)-*N'*-(3,4-dichlorophenyl)-urea, is a polychlorinated phenyl urea pesticide, marketed under the trademark TCC and used primarily as an antibacterial additive in personal care products. Despite its extensive use over several decades, environmental occurrence data on TCC are scarce. This is due in part to a lack of analytical techniques offering the desired sensitivity, selectivity, affordability, and ease of use. This need is addressed here by introducing a liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS) method allowing for the determination of TCC concentrations in aquatic environments at the ng/L level. TCC was concentrated from aqueous samples by solid-phase extraction, separated from interferences on a C_{18} column by either isocratic or gradient elution, and detected and identified in negative ESI mode by selectively monitoring the $(M - H)^-$ base peak (*m/z* 313) and its ^{37}Cl-containing isotopes (*m/z* 315, 317) that served as reference ions. Particulates contained in aquatic samples were extracted and analyzed separately. Accurate quantification was achieved using stable isotopes of TCC and triclosan as internal standards. Addition of 10 mM acetic acid to the mobile phase yielded acetic acid adducts ($[M - H + 60]^-$) that were successfully exploited to boost method sensitivity and selectivity, especially when analyzing challenging environmental matrixes. Method detection limits were matrix dependent, ranging from 3 to 50 ng/L. In 36 grab samples obtained from the Greater Baltimore area, TCC was detected in river water and wastewater at concentrations of up to 5600 and 6750 ng/L, respectively. Raw and finished drinking water did not contain detectable quantities of the pesticide (<3 ng/L). In conclusion, the new LC/ESI/MS method was applied successfully to collect environmental occurrence data on TCC in U.S. water resources. Study results suggest that the bacteriostat and pesticide is a frequent but currently underreported contaminant whose environmental fate and behavior deserve further scrutiny.

Introduction

Triclocarban, *N*-(4-chlorophenyl)-*N'*-(3,4-dichlorophenyl)-urea, is a nonagricultural pesticide released into wastewater in the U.S. at rates of 500 000−1 000 000 pounds per year (*1*). Manufactured overseas and sold under the trademark TCC, the chemical is added for its bacteriostatic properties (*2*) to antimicrobial soap, cosmetics, and other personal care products at levels of up to 5 wt % (*3*). The peer-reviewed literature contains little information on the fate and behavior of TCC in wastewater and none on the environmental occurrence of the chemical in U.S. water resources. The most recent study dates back to 1975 (*4*). Some additional, nonpeer-reviewed data are summarized in two reports recently submitted to the High Production Volume Chemical Challenge Program of the U.S. Environmental Protection Agency (EPA) (*1*, *5*).

Triclocarban has a number of properties suggestive of potential adverse environmental behavior. The chemical is toxic to humans and other animals (reviewed in ref *1*) by triggering methemoglobinemia, a reduction in the rate at which exposed mammals conceive, in the number of offspring born, as well as in the survival rate and body weight of the young. Its polychlorinated aromatic structure suggests a potentially significant resistance to biotransformation and biodegradation (*6*). Indeed, in mineralization experiments, ^{14}C-labeled TCC persisted for 2−4 weeks without appreciable loss when added at levels of 200 μg/L to activated sludge and raw sewage (*4*). In enhanced conditions, the chemical is biodegradable, as demonstrated in the same study using acclimated activated sludge. TCC is not very water-soluble (~11 mg/L at 20 °C) (*5*) and partitions readily into fat (estimated logarithmic octanol/water partition coefficient (log K_{OW}) of 4.2−6.0) (*5*). According to predictions obtained with a computer model (PBT-Profiler; Version 1.203) used by the U.S. EPA, TCC is expected to persist in the environment with a half-life in soil and sediment of 120 and 540 days, respectively. Taken together, these properties imply that TCC is both persistent and susceptible to bioconcentration (*6*), thereby posing potential ecological and human health risks.

The dearth of peer-reviewed environmental occurrence data for this high production volume chemical can be explained in part by analytical challenges relating to the pesticide's structure. Triclocarban is a substituted phenyl urea compound. Its thermal lability and the presence of two active hydrogen atoms in the urea moiety preclude direct analysis by gas chromatography (GC) (*7*). In the past, ^{14}C-labeled TCC has been detected successfully in biotransformation experiments using thin-layer chromatography (TLC) and autoradiography (*4*). Hong et al. employed ether extraction followed by TLC and ultraviolet absorption measurement in methanol at a wavelength of 265 nm, for the quantitative analysis of TCC in whole blood (*8*). Hoar and Bowen determined TCC levels in rat and human blood using acetone extraction followed by TLC cleanup (*7*). Purified extracts were derivatized with *N,O*-bis(trimethylsilyl)acetamide and analyzed by gas chromatography/mass spectrometry (GC/MS). Gruenke et al. (*9*) measured TCC and its major metabolites (2′-hydroxy sulfate and *N*- and *N'*-glucuronides) in urine and human plasma by GC/MS; compounds were extracted from human specimens and hydrolyzed enzymatically and chemically, and the liberated phenylurea moieties were derivatized using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). This labor-intensive approach involved the use of deuterium-labeled internal standards and facilitated accurate determination of TCC and its hydroxylated derivatives at the ng/mL range in biological media (*9*).

The present study concentrated on the development of a liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS) technique with the goal of lowering

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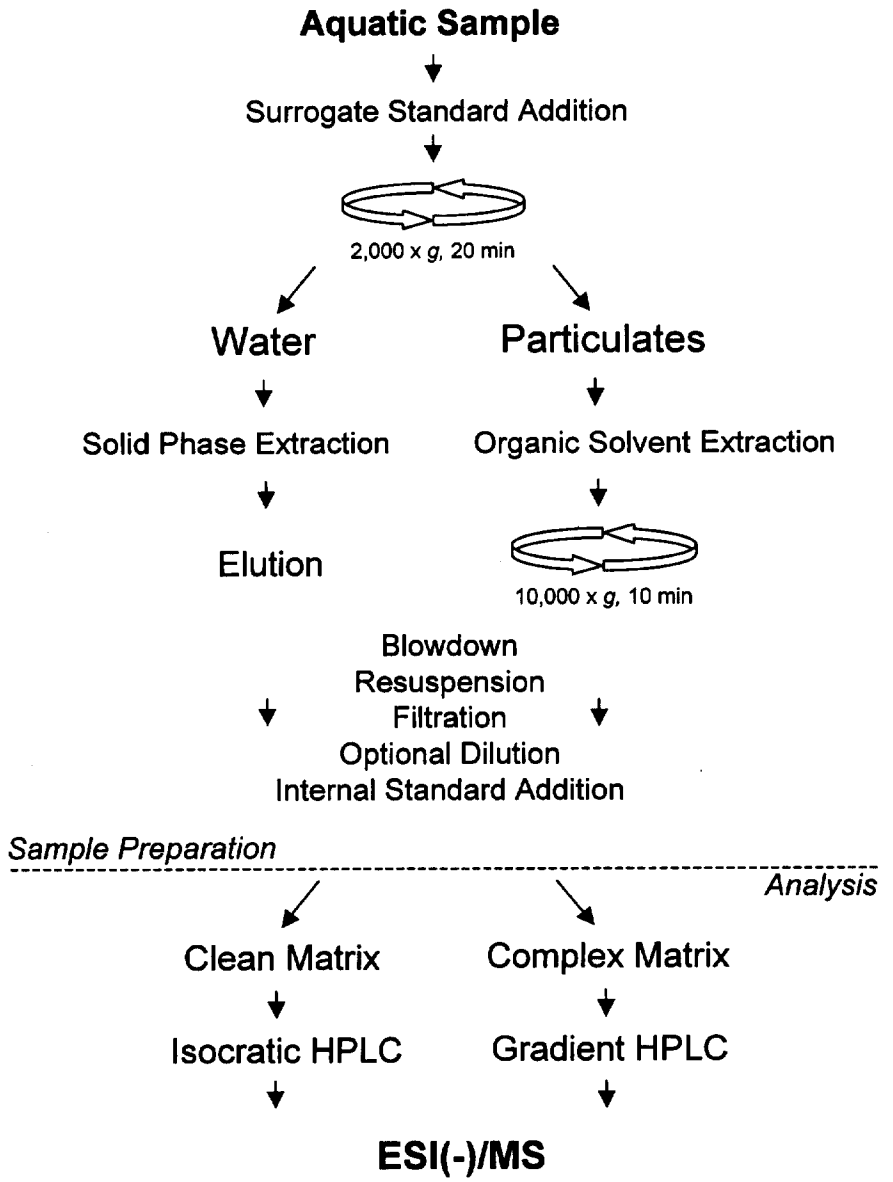

FIGURE 1. Sample preparation and analysis strategy for the determination of triclocarban in aquatic samples using isocratic and gradient high-pressure liquid chromatography (HPLC) followed by electrospray ionization mass spectrometry in negative mode (ESI(−)/MS).

previously achieved detection limits and facilitating the rapid, sensitive and selective detection and quantification of underivatized triclocarban in aquatic samples at the ng/L level.

Experimental Procedures

Chemicals. Triclocarban [101-20-2] was obtained from the Aldrich Chemical Corporation (Milwaukee, WI). Deuterated triclocarban (TCC-d_7; Figure 2B; 99%) was a gift from Cambridge Isotope Laboratories Inc. (Andover, MA). Isotope labeled triclosan ($^{13}C_6$-TCS; Figure 2C; 98.7%), containing a uniformly labeled 2,4-dichlorophenoxy ring, was a gift from Ciba Specialty Chemicals (Basel, Switzerland). All analytical solvents (HPLC grade or better) were purchased from Acros Organics (Fairlawn, NJ). All other chemicals were purchased from the Sigma-Aldrich Company (Milwaukee, WI) and were of the highest purity available.

Sampling. Water samples were collected in duplicate using disposable, precleaned 1 L sample bottles. Trip blanks consisted of 1 L bottles filled with reagent water (18.2 MΩ resistance) obtained from a Nanopure Diamond Ultrapure water system (Barnstead; Dubuque, IA). The sampling period for this study ranged from September 2002 through November 2003. Raw and finished drinking water samples were obtained from two drinking water treatment plants in Baltimore. Additional wastewater influent and effluent samples were collected at the Back River wastewater treatment plant (WWTP) on two occasions. No measurable precipitation was recorded at the sampling locations in the 12 h period preceding the sampling events. Additional sampling locations included three residential wells in Baltimore County (groundwater samples) and 19 locations along six urban streams in the Greater Baltimore area (Gwynns Falls, Gwynns Run, Jones Falls, Maidens Choice Run, Stoney Run, and Western Run). Prior to the collection of residential well and drinking water samples, point-of-use water filters were removed as needed, and the faucets were allowed to run for several minutes to avoid capture of stagnant water.

All urban stream sampling locations were located upstream of WWTP inputs. However, some locations were chosen strategically based on previous reports of wastewater infiltration from leaking sewer systems. All samples were immediately placed on ice, shipped to the laboratory, fortified with isotope-labeled standards (500 ng/L of TCC-d_7 and/or $^{13}C_6$-TCS), and stored at −20 °C as needed. All analytical data reflect average concentrations of two independent measurements of duplicate samples processed in parallel.

Sample Preparation. Water samples were spun at 2000*g* for 20 min to remove solids. The supernatant was passed through an Oasis solid-phase extraction (SPE) cartridge (hydrophilic−lipophilic balance (HLB), 3 cm^3/60 mg sorbent; Waters Corp., Milford, MA) and eluted with organic solvents (4 mL, 50:50 methanol/acetone containing 10 mM acetic acid). Eluates were dried, reconstituted (1 mL, 50:50 methanol/acetone), filtered (0.2 μm PTFE, 13 mm syringe filters; Nalge Nunc Int., Rochester, NY), reduced to initial eluent strength by dilution, and analyzed by LC/ESI/MS. Sample particulates harvested by centrifugation were extracted overnight with organic solvents (2 mL, 50:50 methanol/acetone), dried and processed as described previously. Raw sewage (40 mL) was diluted (250 mL final volume) and spun at 10000*g* for 10 min. The complete sample preparation and analysis process is summarized in Figure 1.

LC/MS Analysis. Chromatography was carried out on an Ultra IBD C_{18} column (5 μm particle size, 2.1 × 150 mm; Restek Corporation, Bellefonte, PA) using a DGU-14A eluent degaser, two LC-10ADvp gradient pumps, and an SCL-10Avp system controller (Shimadzu Corporation, Columbia, MD). Samples were injected (10−100 μL) with an autosampler (Shimadzu SIL-10ADvp) controlled by LCMS Lab Solutions software (v2.04). An isocratic method was used for samples lacking noticeable turbidity (0.2 mL/min; 70% acetonitrile, 30% water, 10 mM acetic acid). Total analysis time was 9.5 min with analytes eluting at 4.8 min ($^{13}C_6$-TCS) and 7.0 min (TCC; TCC-d_7). Compounds were detected and quantified using a quadrupole mass spectrometer (Shimadzu LCMS 2010) in negative ESI mode. The curved desolvation line (CDL) and Q-array were set to 35 and −5 V, respectively. Block and CDL temperatures were 220 and 230 °C, respectively. Nitrogen desolvation gas was flowing at 4.2 L/min. Quantitative analysis was performed in selective ion monitoring mode (SIM). Positive identification of analytes was based on three criteria: (a) elution within the expected retention time window (RT ± 0.1 min), (b) detection of the characteristic $(M - H)^-$ base ion of triclocarban (*m/z* 313) or its acetic acid adduct (*m/z* 373), and (c) detection at the anticipated intensity of a minimum of one or more naturally occurring ^{37}Cl-containing isotopes (*m/z* 315, 317; *m/z* 375, 377) that served as reference ions for triclocarban and the triclocarban adduct, respectively. Samples having noticeable turbidity such as raw sewage were analyzed by using a linear gradient method (0.2 mL/min; 20 min) running from 25 to 100% acetonitrile; in some instances, the eluent was amended with 10 mM acetic acid as discussed later. For samples processed before the TCC-d_7 standard became available, recovery was calculated and corrected for by using the $^{13}C_6$-labeled internal standard. The validity of this approach was checked periodically using matrix spikes and the method of standard additions as described previously (*10*). Quantification was performed using a linear calibration and a minimum of seven calibration levels. During routine analysis of environmental samples, instrument precision and accuracy were assessed every fourth sample by measuring a 100-ppb quality control standard using a tolerance cutoff value of ±20%. Additional quality assurance and quality control protocols were followed as described previously (*11*).

Results and Discussion

LC/ESI/MS Method Development. The sample preparation strategy used for TCC analysis is shown in Figure 1. It was designed to yield information on both the total TCC concentration in the sample and the compound's partitioning behavior in the environment studied. Aquatic samples arriving in the laboratory were spiked with isotope-labeled surrogate standard and allowed to equilibrate. Thereafter, samples were separated by centrifugation into aqueous and solid fractions, which underwent solid-phase extraction and organic solvent extraction, respectively. Following addition of an optional internal standard just prior to analysis, total TCC concentrations in environmental samples were calculated from the combined analyte mass recovered from aqueous and solid fractions.

The ionization behavior of TCC was explored as a function of ESI polarity, CDL voltage, solvent type, and modifier added to the eluent. Flow injection of 10 ng of TCC into the quadrupole mass spectrometer operating in scan mode yielded a detectable signal only in negative ionization mode. No compound fragmentation was observable regardless of CDL voltage settings, thereby restricting detectable ions to the monoisotopic base peak at *m/z* 313 ($[M - H]^-$; $^{35}Cl_3$-TCC; 100% relative theoretical abundance); and its chlorine-37 containing molecular isotopes, *m/z* 315 ($^{35}Cl_2/^{37}$Cl-TCC; 97.5%), *m/z* 317 ($^{35}Cl/^{37}Cl_2$-TCC; 31.7%), and *m/z* 319 ($^{37}Cl_3$-TCC; 3.4%). Because of the relatively low abundance of $^{37}Cl_3$-TCC, only the three most prominent natural isotopic $(M - H)^-$ base ions were chosen for compound identification (*m/z* 313 and 315), confirmation (*m/z* 317), and quantification (*m/z* 313) in SIM mode.

In a screening study of solvents and additives, a mixture of acetonitrile, water, and acetic acid was selected as the preferred mobile phase yielding optimal signal intensities and signal-to-noise (S/N) ratios in the mass range of interest. Use of the volatile modifier (acetic acid) at 10 mM final concentration caused a notable overall increase in signal intensity and yielded additional characteristic target ions in the form of TCC/acetic acid adducts at *m/z* 373, 375, and 377 for nonlabeled TCC (Figure 2A) and at *m/z* 380, 382, and 384 for the deuterated standard, TCC-d_7 (Figure 2B). The modifier also enhanced the signal intensity of $^{13}C_6$-TCS considerably; however, in contrast to TCC, adduct formation with $^{13}C_6$-TCS was negligible (<10%; Figure 2C). Since the formation of adducts was not 100% efficient, additional diagnostic reference ions were obtained from free TCC at *m/z* 313 and 315.

The stability of TCC adduct formation was explored as a function of analyte concentration by monitoring the adduct ion at *m/z* 373, $[M - H + 60]^-$ (Figure 3A). In the range of interest from 0 to 5000 pg on-column, the absolute signal intensity increased linearly with TCC concentration, thereby routinely yielding calibration curves with R^2 values of ≥0.999. The calibration curve shown in Figure 3B was obtained by eluting TCC from a C_{18} column using an isocratic aqueous mixture consisting of 70% acetonitrile and 10 mM acetic acid. For the analysis of highly contaminated environmental samples, a gradient method was developed in which the acetonitrile content was raised linearly from 25 to 100% over a period of 20 min. The average retention time of TCC for isocratic and gradient elution was 7.5 and 15.5 min, respectively. Nonlabeled and deuterated TCC eluted together with minimal separation being observed under the conditions tested.

Method Performance. The sensitivity of TCC analysis was investigated as a function of chromatography conditions and target ions, by analyzing via on-column injection seven replicates of fortified drinking water samples. Limits of detection for TCC were estimated according to the Code of

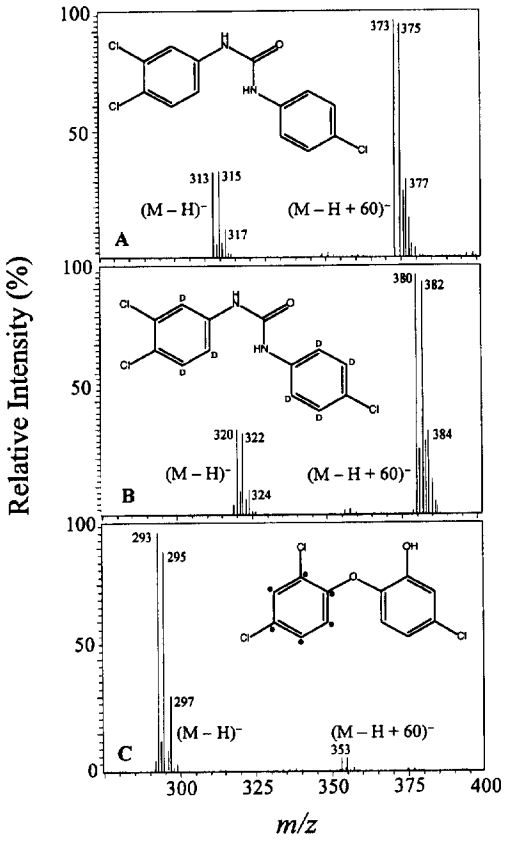

FIGURE 2. Representative mass spectra obtained by flow-injection of 10 ng of analyte into the quadrupole mass spectrometer scanning in the *m/z* range of 275–400. The $(M - H)^-$ base ion and its acetic acid adduct ion ($[M - H + 60]^-$) were detectable when using as eluent a mixture containing acetonitrile (70%), water (30%), and acetic acid (10 mM). Significant adduct formation was observed with triclocarban (A) and deuterium-labeled (d_7) triclocarban (B) but not with the internal standard, $^{13}C_6$-triclosan, containing a uniformly labeled 2,4-dichlorophenoxy ring (C).

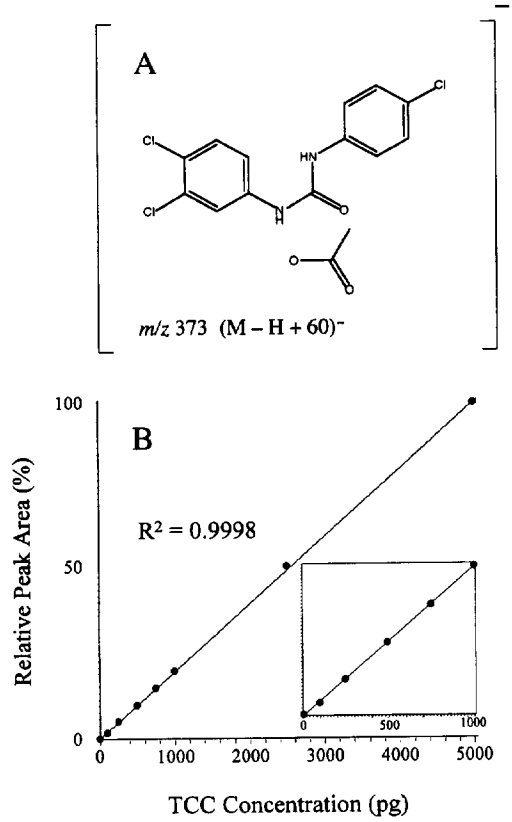

FIGURE 3. Intensity of the triclocarban/acetic acid adduct signal (A) at *m/z* 373 ($[M - H + 60]^-$) increased linearly with triclocarban mass in the range of 0–5000 picogram (pg) on-column. Accurate quantification was achieved by using linear calibration curves that typically had R^2 values of ≥0.999 with the intercept near the origin (0,0) without forcing (see panel B; the inset magnifies the calibration region near the origin). The location of proton loss in panel A is hypothetical and for illustrative purposes only.

Federal Regulations (*12*). Results are presented in Table 1 together with detection limits reported by other investigators if available. The isocratic method yielded lower detection limits (0.21 and 0.06 ng on-column for $(M - H)^-$ and $(M - H + 60)^-$, respectively) than the gradient technique (1.9 and 0.24 ng) regardless of the target ion chosen for quantitation. Addition of 10 mM acetic acid to the mobile phase and monitoring of TCC/acetic acid adducts increased the sensitivity of isocratic and gradient analysis by a factor of 3.5 and 7.9, respectively (Table 1). Compared to previously published protocols summarized in Table 1, the major benefits of LC/ESI/MS analysis are the convenience of detecting TCC itself without the need for compound derivatization, as well as a gain in sensitivity by up to 3 orders of magnitude (Table 1). Two previously published methods rival the performance of LC/ESI/MS. A gas chromatography/electron impact/mass spectrometry protocol (GC/EI/MS) was developed for the determination of TCC in blood and urine (*9*), and a liquid chromatography tandem mass spectrometry method (LC/MS/MS) was developed for the analysis of the mothproving agents (Mitins) sulcofuron and flucofuron in river water (*13*). However, the former method required extensive sample cleanup and derivatization (*9*), whereas the latter protocol was geared to other analytes and employed TCC only as an internal standard (*13*). In both cases, TCC detection limits were not reported.

To validate the LC/ESI/MS method for quantification of TCC in environmental samples, spike recovery experiments were conducted with water samples previously determined to contain no detectable quantities of the analyte. In these experiments, the average percent recovery was 103 ± 6% relative standard error. Standard addition experiments were conducted with wastewater samples previously determined to contain TCC. Recovery rates in these experiments averaged 95 ± 9% relative standard error.

Method Application to Environmental Monitoring. The LC/ESI/MS method was used to determine TCC concentrations in aquatic samples from the Greater Baltimore area, MD. The benefit of pairing gradient chromatography with monitoring of TCC/acetic acid adducts is illustrated in Figure 4A–F. A diluted extract of raw wastewater was analyzed by both isocratic LC/ESI/MS (Figure 4A) and gradient LC/ESI/MS (Figure 4D) first in the absence and then in the presence of 10 mM acetic acid in the eluent. Monitoring of $(M - H)^-$ ions of TCC with the isocratic method did not yield a quantifiable peak (S/N ratio of 0.3; Figure 4B). Targeting $(M - H + 60)^-$ adduct ions in the presence of acetic acid provided a better signal, although a lack of specificity was still evident (S/N ratio of 19; Figure 4C). Analysis of the same sample with the gradient method in the absence of the modifier allowed for the partial separation of TCC from contaminants sharing the ion at *m/z* 313 (Figure 4E). However, the best results were achieved by gradient chromatography targeting the adduct of TCC and acetic acid. This approach yielded a

TABLE 1. Comparison of Analytical Methods for the Detection of Triclocarban[a]

method	matrix	detection limit [ng]	derivatization	ref
GC/FID	synthetic samples	20	TMS	14
GC/MS	plasma, urine	NA	TMS	9
GC/ECD	blood	25	TMS	7
TLC/UV	blood	250	none	8
QTLC/UV	deodorant	NA	none	15
PB-LC/MS	WWTP effluent	NA	none	16
LC/MS/MS	water (IS only)	NA	none	13
LC/MS (isocratic LC)	drinking water, river water		none	this paper
m/z 313 ($[M - H]^-$)		0.21[b]		
m/z 373 ($[M - H + 60]^-$)[c]		0.06[b]		
LC/MS (gradient LC)	river water wastewater		none	this paper
m/z 313 ($[M - H]^-$)		1.9[b]		
m/z 373 ($[M - H + 60]^-$)[c]		0.24[b]		

[a] Abbreviations: GC, gas chromatography; LC, liquid chromatography; (Q)TLC, (quantitative) thin-layer chromatography; FID, flame ionization detector; MS, mass spectrometry; ECD, electron capture detector; UV, ultra violet; PB, particle beam; TMS, trimethylsilylation; IS, internal standard; NA, not available. [b] Detection limits were determined according to CFR 40, part 136, Appendix B; seven replicates of a low-level standard were analyzed, and the detection limit was estimated by multiplying the calculated standard deviation by 3.143, the $t_{0.99}$ value of the *t*-distribution; reported values are for drinking water; method detection limits were a function of the sample matrix as shown in Figure 5A. [c] Analyzed in the presence of 10 mM acetic acid in the eluent.

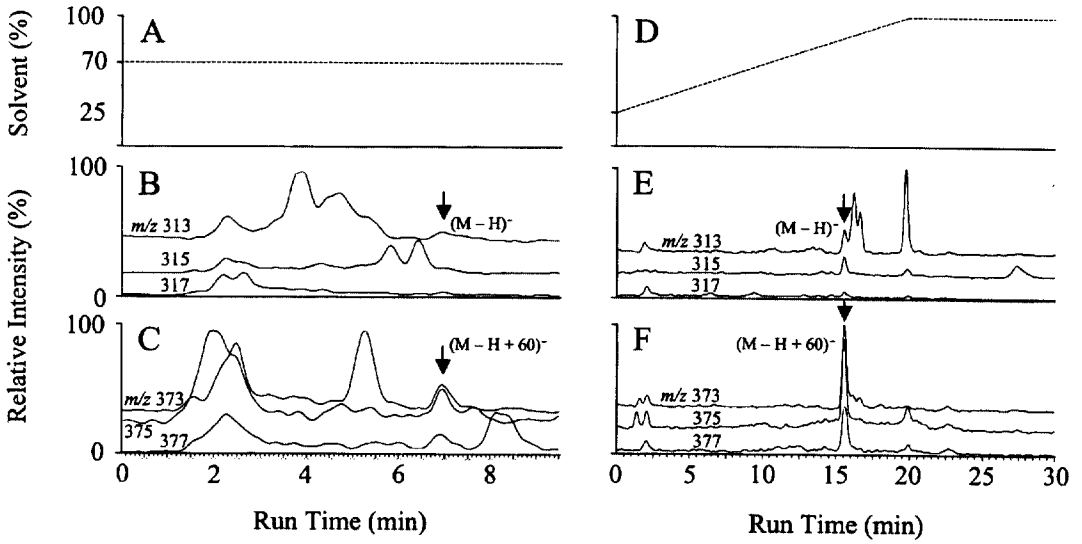

FIGURE 4. To achieve accurate triclocarban analysis in complex matrixes such as the diluted raw wastewater extract shown here, the isocratic LC/ESI/MS method (left panels) was replaced with a gradient approach (right panels). Elution of triclocarban from the C_{18} column in an isocratic mixture of acetonitrile and water yielded no quantifiable triclocarban peak when monitoring the $(M - H)^-$ base ion at *m/z* 313 and its two most prominent natural isotopes at *m/z* 315 and 317 (B). Addition of 10 mM acetic acid to the eluent allowed for the detection of triclocarban/acetic acid adduct ions at *m/z* 373, 375, and 377, as indicated by the arrow; peak shapes and signal intensities were still poor, however (C). Gradient elution (D) with a mixture of acetonitrile and water allowed for partial separation of triclocarban from coeluting interferences at *m/z* 313 (E). Addition of 10 mM acetic acid to the eluent caused further significant improvements in both signal intensity and signal-to-noise ratios by moving the target analyte into a more quiet mass range via formation of acetic acid adducts ($[M - H + 60]^-$) (F).

significantly improved S/N ratio of 38 versus 6.4, by raising the quantitation ion by 60 atomic mass units from *m/z* 313 into a quieter mass region (*m/z* 373; Figure 4F). Noise at *m/z* 313 was observed consistently in the time window of TCC elution when analyzing wastewater and contaminated urban stream samples. By targeting TCC adduct ions, the potentially detrimental effect of these unidentified interferences on TCC analysis was successfully circumvented.

The histogram in Figure 5A summarizes the distribution of estimated method detection limits and measured TCC concentrations in aqueous samples from the Greater Baltimore area. All drinking water samples tested negative for TCC. The detection limit, defined as the minimum analyte concentration that can be reported at the 99% confidence level as being greater than zero (*12*), was 3 ng/L in raw and finished municipal drinking water (Figure 5A; samples 1–5). The method detection limit for groundwater from residential wells was estimated at 20 ng/L (Figure 5; samples 6–8), based on the concentration needed to reach a S/N ratio of 3:1. Nine of 26 urban stream samples tested negative for TCC at an estimated detection limit of 25–30 ng/L (Figure 5; samples 9–17). Seventeen river samples, obtained from various locations along six urban streams, tested positive for TCC. Measured concentrations spanned more than 2 orders of magnitude, ranging from 33 to 5600 ng/L. Influent samples from a local wastewater treatment plant, obtained on two occasions in September 2002 and November 2003, contained TCC at 6650 and 6750 ng/L, respectively. Since all urban sampling stations were located upstream of wastewater treatment plant inputs, the detection of TCC in river water

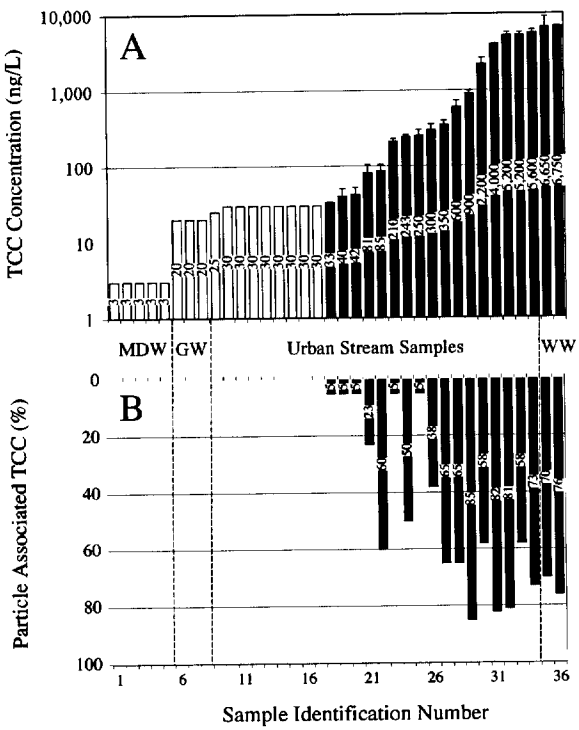

FIGURE 5. Histogram (A) showing detection limits (empty bars) and environmental concentrations of triclocarban (solid bars) determined by LC/ESI/MS in 36 aquatic samples from the Greater Baltimore area, representing municipal drinking water (MDW; samples 1–5), groundwater from three residential wells (GW; 6–8), six urban streams (samples 9–34), and raw wastewater (WW; samples 35 and 36) captured at the intake of the Back River treatment plant. Reported values represent average concentrations of duplicate samples processed in parallel; error bars indicate the higher of two measurements. Surrogate standard, added to the sample prior to centrifugation, allowed for the determination of particle-associated triclocarban, expressed as a fraction of the total analyte mass detected in the sample (B).

was interpreted as an indication of raw wastewater inputs from leaking sewer lines. The 90-year old sewer system of Baltimore City is known to leak wastewater in many locations, requiring upgrades at costs estimated at $1.3 billion (*17*). Stormwater runoff does not contribute to the load of antimicrobials unless it contains flow volume from sewer overflows.

Under ambient conditions in the environment, TCC exists as a nonionic compound having a logarithmic octanol/water partitioning coefficient (log K_{OW}) estimated at 4.2–6.0 (*5*). Accordingly, the compound is expected to sorb readily to particulate organic matter and to nonfilterable colloidal matter. The environmental partitioning behavior at ambient pH in river water and raw wastewater was determined by analyzing organic solvent extracts from sample particulates harvested by centrifugation (Figure 1). The histogram in Figure 5B summarizes the relative fraction of TCC associated with sample particulates expressed as a percentage of the total analyte mass detected in a given sample. From the plot, it is evident that in samples containing more than 300 ng/L of TCC, the majority of the analyte mass was present as a sorbate rather than a solute. Due to weighing errors, etc., the accuracy of analysis is low when analyzing small particulate fractions. Therefore, the percentages reported for samples 18–26 have a higher degree of uncertainty relative to those reported for samples 27–36. On the basis of the higher quality data for stream samples 27–34, it is estimated that, on average, 72 ± 10% of the total TCC mass in natural surface waters is sorbed to the experimentally defined particulate fraction.

The present study provides the first peer-reviewed environmental occurrence data for TCC in U.S. drinking water and river water. Additional unpublished and nonpeer-reviewed data are summarized in two reports recently submitted by the chemical industry to the U.S. EPA's High Production Volume Chemical Challenge Program (*1*, *5*). Environmental concentrations of TCC summarized therein range from nondetectable in U.S. drinking water (<10 ng/L), to 240 ng/L in U.S. surface waters, to 50 000 ng/L in wastewater (*5*). Compared to previously reported data for surface waters in the United States (*5*), the maximum TCC concentrations detected in surface waters of the Greater Baltimore area are 20-fold higher. This discrepancy may be due to a number of factors including (i) increased usage of TCC in recent years, (ii) underestimation of concentrations in previous studies resulting from the use of less selective techniques (e.g., HPLC/UV), (iii) regional differences in TCC usage translating into geographically distinct environmental concentrations, (iv) sampling in locations that were less impacted by inputs of raw and treated wastewater, and (v) underestimation of true concentrations due to the analysis of filtered water, thereby disregarding the particle associated mass of TCC.

The isocratic and gradient LC/ESI/MS methods presented here may serve as valuable research tools for obtaining additional data on the occurrence of TCC in the environment. Use of a mixed-mode copolymeric solid-phase extraction resin for sample preparation ensures that the described analytical method is readily extendable to include additional pharmaceuticals and personal care products as well as caffeine and other markers of sewage contamination. Considering the significant persistence of TCC in wastewater (*4*) and the chemical's widespread use at rates approaching one million pounds per year in the U.S. alone (*1*), further studies are needed to both determine the fate and behavior of TCC in the environment and to elucidate potential human health and ecological risks associated with the environmental occurrence of this persistent polychlorinated phenyl urea compound.

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